

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE New 3'-5' exo nuclease Mutant of *T. thermophilus*

132

Fr m Page No. \_\_\_\_\_

4/13 -

Purpose: ~~See~~ Previous clone (P. 129) of a 3'-5' exo nuclease mutant of *Thermotoga neopolitana* (Tn) proved not to ~~produce~~ overexpress a heat sensitive or heat polymerase activity. Therefore Dels ~~proceeded~~ made a new clone. The purpose of his experiment is to screen pre + post heat kill for a polymerase activity. If activity is more thermostable then proceed w/ a PET + (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> ppt.

3 grams of cells - resuspend in 4ml of crack buffer -  
 Sonicate with ~~mini tip~~ micro tip ~~last 20 seconds~~

crack buffer -  
 20mM Tris pH 7.5  
 10mM KCl  
 .1mM EDTA  
 5mM Bme  
 5% glycerol

#575 - .824 before crack  
 A575 - .198 after crack 6x 20sec  
 76% crack - minotip C  
 setting 4.

Save 400µl → No heat treatment

Aliquot the rest of the cracked material to 2mL eppendorf  
 heat kill 10min → temp. @ 80° - 90° C -  
 note: temperature rose to > 90 in maybe up to 5min

Spin in microfuge @ 14000g 30 minutes -

heat treat supernatant < 90° > 85° C - in 5 minutes -  
 spin in microfuge @ 14000 x g 10 minutes -

Witnessed & Understood by me,

May Jorge

Date

6/20/95

Invented by

Recorded by

Date

6/13/95

T Page 1

age N \_\_\_\_\_

06/13/95

say in thermostable polymerase activity -

mix - TAQ premix - premade by A.G. -

add 1.1  $\mu$ l / 500  $\mu$ l premix  
of 2320 dCTPDilution  $\frac{5}{495}$  -  $\frac{10}{90}$  -  $\frac{1}{1000}$   
 $\frac{10}{290}$  -  $\frac{1}{3000}$  $\frac{1}{1000}$  1  $\mu$ l  
2  $\mu$ l heat treated $\frac{1}{3000}$  1  $\mu$ l  
2  $\mu$ l $\frac{1}{1000}$  1  $\mu$ l  
2  $\mu$ l before heat treatment $\frac{1}{3000}$  1  $\mu$ l  
2  $\mu$ l mistake made - put 78  $\mu$ l of premix should have only used 48 !!incubate 10' @ 74°C in a heated water block - quench run w/  
10  $\mu$ l of .5M EDTA - spot 30  $\mu$ l on 6F/C 11.0i

Wash filters

1x 10% TCA 5'

3x 5% TCA 3'

2x 5% TCA

dry + count in econofluor LSF

AM CPM1

1	$\frac{1}{1000}$	2994.00
2		2384.00
3	$\frac{1}{2000}$	622.00
4		888.00
5	$\frac{1}{1000}$	3612.00
6		5296.00
7		1234.00
8	$\frac{1}{2000}$	1662.00
9		964.00
10		90094.00
11		89736.00
12		89120.00
13		40.00

H.Kill

No H Kill

S.A

27 Oct 95

First approx. - looks as though not  
as much activity after  
heat & kill - need to do  
less dilutions to in order  
to ascertain what exactly  
is going on.

Seeing host polymerase act. in No Heat Kill?

Repeat w/  $\frac{1}{500}$   
 $\frac{1}{200}$  dil's -

mz

0/20/95

To Page No. \_\_\_\_\_

Used &amp; Understood by me,

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